

The University of Chicago

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OF THE GONADOTROPIC ACTIVITY OF
THE PITUITARY GLAND BY VARIOUS
EXTRACTS AND PROCEDURES

A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES
IN CANDIDACY FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY

DEPARTMENT OF BIOCHEMISTRY
DECEMBER, 1943

By
GEORGE W. BEACH

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Gonadal control of the gonadotropic activity of the anterior lobe of the pituitary gland is well established by numerous studies, but the actual mechanisms involved have not been elucidated. A detailed quantitative consideration of the early studies on the effects of gonad extracts on the anterior pituitary body of the castrated animal cannot be undertaken here. This has been covered in quantitative detail in a previous review (1). The well established facts are: (a) gonadectomy leads to histological changes in the anterior pituitary, (b) gonadectomy causes an increased urinary excretion of gonadotropins and a higher concentration of gonadotropins in the anterior lobe of the pituitary, (c) after puberty the normal male's pituitary contains a higher concentration of or secretes gonadotropins at a higher rate than that of the normal mature female, but before puberty the hypophyses are undifferentiated sexually.

Prevention of the histological changes.--It is well established that pure androgens and estrogens if given in sufficiently large doses prevent the appearance of these changes in the anterior lobe of the castrated rat. The estrogens are much more potent in this respect than the androgens and castrated males require much higher doses of either steroid type than the spayed females. The fact that relatively high doses of the steroid hormones are required in every case and that relatively small amounts of very crude water extracts or suspensions of testis tissue appear to be equally effective and may even show some sex specificity, raises the question whether the only inhibitors of pituitary gonadotropic activity are the known steroid sex hormones or whether other gonadal secretions are involved. So far the histological approach does not appear to have answered this question satisfactorily.

Increased gonadotropic secretion, concentration, and excretion in the gonadectomized animal.--This curious phenomenon appears paradoxical. The findings suggest that after removal of

the gonadal control by castration the pituitary secretes gonadotropin more rapidly than normally or that as a result of the loss of gonad tissue, the gonadotropins are not utilized or destroyed, hence a plethora of blood gonadotropins follows and this leads to the increased urinary excretion and increased storage in the pituitary. It is, of course, not known whether the gonads actually alter gonadotropins when stimulated thereby, but it is not impossible that the normal gonads may actually modify gonadotropin to a type of pituitary inhibitor and thus control the pituitary activity. If so, implants or cell suspensions of gonads might prove to be good inhibitors and there might be striking species and sex differences or specificities. Up to the present the most convenient method for studying these changes is to determine the gonadotropin content of the pituitaries of normal, treated and untreated castrated animals. Until recently this was done by implants of the pituitary, a method which has definite limitations from a quantitative point of view.

In this study we used saline suspensions of the finely ground pituitary tissue. It is difficult to predict what one should expect from the pituitaries of rats treated in various ways. The simplest result one might expect is a lowering in the rate of formation and hence of the gonadotropin content of the pituitary which it was our aim to inhibit. If however the inhibition is limited only to the actual secretion and not to the production of gonadotropin as well, then we might expect a storage of gonadotropins and hence a higher concentration. It is also possible that inhibition of the gonadotropic activity of the pituitary involves a decrease in the rate of formation as well as of secretion of gonadotropins. This might result in no change in the concentration of gonadotropin in the gland.

Age and sex differences.--The fact that the pituitary before puberty is low in gonadotropin content and rate of secretion must mean that the pituitary has not been "activated" by gonadal secretions. We know that the gonads in these immature animals are readily stimulated by injected gonadotropins and we also know that the accessory sex organs of these immature animals are easily stimulated by androgens and estrogens. Apparently the immature pituitary must first be "activated" by gonad secretion and it appears that the nature of the gonadal secretion determines whether the male or female type of pituitary is evolved. In other

words in the normal immature animal we have all the evidence of castration if we limit our observations to the accessory sex organs, but if we examine the pituitaries there is no evidence of the castration effects observed in the adult. Presumably we are justified in saying that the pituitary of the immature does not require as high a blood concentration of androgen or estrogen to inhibit its secretion of gonadotropin or that some other factor is involved. This factor need not, of course, be of gonad origin and may not be the same as the factor which normally controls the gonadotropic activity in the adult.

The more recent studies which have a direct bearing on the quantitative and qualitative aspects of the general problem before us are: (a) the inhibition of estrus in the normal rat, (b) the antagonistic action of estrone on comb growth in the cock, (c) parabiosis studies on castrates, and (d) parabiosis studies with X-ray sterilized males.

Inhibition of estrus as a method for studying the inhibition of gonadotropin activity.--If the control of the gonadotropic activity of the anterior pituitary is normally due to the sex hormones in the blood then one would expect low concentrations to be effective. Likewise, if the "antagonistic" action of androgens in normal females acts through the pituitary one would expect androgens to inhibit the estrous cycle. In the earliest studies of this type it was found that very crude extracts in low doses did arrest the cycle in the rat. Later, crude lipin concentrates containing approximately one international unit of androgen activity per dose also arrested the estrous cycle in the rat. However, careful fractionation of this crude concentrate into the non-androgenic phospholipin and the more concentrated androgenic fractions led to the finding that the estrus inhibiting activity, in terms of the equivalent of the original dosage of the crude lipin concentrate, was found in the phospholipin fraction and not in the more refined androgenic fraction. An equivalent dosage of testosterone propionate expressed in terms of comb growth activity in the capon also did not arrest the cycle, but thirty times that dose of pure testosterone propionate did inhibit the cycle. It should also be noted that pure lecithin in comparable doses and prepared from other tissues including hog ovaries, also arrested the estrous cycle (2).

It is obvious then that high doses of pure androgens cause

inhibition of the estrous cycle, but it is not certain that the path of action is limited to the pituitary and thus indirectly through control of the ovary to the vagina. The facts are that the dosage of androgen employed actually lowers the vaginal smear response in spayed rats to carefully standardized levels of estrone (3, 4). Hence this method of assay may measure only the inhibition of the end organ response and may not involve the pituitary or gonads at all.

The antagonistic action of estrone on comb growth in the cock.--It is well known that various estrogens lower the rate of comb growth in cockerels and cocks. However, the action in this case is also not limited to the inhibition of the pituitary as a gonad stimulant. The facts are that estrone has a very marked comb growth retarding action to carefully standardized doses of androgens in the capon. This action is so striking and particularly when applied directly to the comb, that we are forced to the conclusion that the action of the estrone is in the main on the comb directly and that the pituitary → gonad → comb path is not necessary (5).

From these "antagonism" studies we conclude that the pituitary may not be involved at all, but that the action directly on the end organ is the most important one. Careful assays on the gonadotropin content of the pituitaries from the spayed rats and capons treated with androgens and estrogens respectively in these antagonism studies are needed.

Parabiosis studies on castrated rats. When a castrate male or female is joined parabiotically with a normal male or female the observations of some (6, 7) are that the gonads and accessory sex organs of the normal twin are stimulated, but that the accessory sex organs of the castrate mate are not stimulated. The interpretation is that as a result of the gonadectomy the hyperactive pituitary secretion acts on the gonads to produce higher rates of secretion of sex hormones, but that the latter in turn do not enter the blood stream of the castrated mate in sufficient concentration to inhibit the pituitary or affect the accessory sex organs. The results from these studies indicate that the pituitary of the castrated male is secreting at a higher rate than that of a castrated female and that it acts mainly as a follicle stimulating factor. The pituitary of the spayed female acted mainly as a luteinizing factor. The striking effects on the gon-

ads and accessory sex organs observed in these parabiotics are reported to be prevented (6, 7) by the injection of very small amounts of testis tissue pulp into the castrated mate. However, no progress has been made in elucidating the character of the active substance in these suspensions. It is very unlikely that the traces of androgens introduced are responsible for the inhibition because the accessory sex organs of the gonadectomized mate are not stimulated. In fact it has been shown that when sufficient androgenic material is injected into the castrated mate the hyperactivity of its pituitary as evaluated by the state of the accessory organs in the normal mate, can be inhibited. This dose has been reported to be 30 μ testosterone propionate per day (8) or the equivalent of approximately 150 grams fresh testis tissue.

Parabiosis studies on X-ray sterilized rats.--When an X-ray sterilized male rat is joined parabiotically with a normal female a continuous estrus is induced (9). However, the accessory sex organs of the X-rayed mate may actually be hypertrophied, showing that the hyperactive pituitary is acting on the internal secreting elements of the X-rayed testes and that in spite of this higher than normal secretion of androgens the pituitary was not inhibited. Others have reported that X-ray sterilization produces castration changes in the pituitary similar to castration (10).

Negative findings.--There are numerous negative reports from direct searches for a non-androgenic pituitary inhibiting factor in testis tissue. Moore and Rubin (11) contend that all the internal secretions involved in the control of the pituitary gonadotropic activity have been isolated. They find no effect on prostate and seminal vesicle weights in normal male rats by feeding daily 0.2 to 1.2 grams of desiccated testis tissue for 3 months. Negative results were obtained by the daily oral administration of 0.2 to 0.6 gram of the same material to normal female rats for a period of 3 to 4 months. The negative results were based on the fact that the vaginal smears remained normal during 3 weeks after approximately nine weeks of treatment. Daily injections of 1 c.c. of an aqueous extract of bull testis tissue, equivalent to 25 grams fresh tissue for 3 weeks resulted in a decrease in the seminal vesicle and prostate weights in normal rats. From these studies the authors concluded that it is unlikely that a specific pituitary inhibiting water soluble hormone is present in testis tissue. These conclusions do not seem to be entirely

warranted. The studies were limited entirely to the reproductive tract and in no case were the pituitaries examined. No observations were made on castrated animals nor upon X-ray sterilized rats.

It must also be granted that negative results may mean nothing. The proper procedure for detection, extraction, and administration may not have been found. Let us recall that for many years negative results were obtained in the search for an extract containing the male hormone.

From a consideration of the brief review above we may be justified in asking the following questions:

(a) In view of the need of relatively high doses of pure androgens and estrogens to inhibit the over-activity of the pituitary of the castrated rat as measured by various methods, are we to conclude that the sex hormone content of the blood of normal animals is involved in any way in this control in the non-castrated animal?

(b) If such low concentrations of the sex hormones are involved in this control in the normal animal in what form are they most effective in the control of the pituitary?

(c) Is a definite ratio of various androgens and estrogens exceptionally effective or do the conjugated, water soluble excretory forms happen to be particularly effective in inhibiting the pituitary?

(d) How can we explain the studies on X-ray sterilized male rats without concluding that a non-androgenic mechanism must be involved?

EXPERIMENTAL

While we did not attempt to answer all of these questions it was hoped that a direct biochemical study of the pituitaries of normal, castrated and X-ray sterilized rats, treated or untreated, might lead to a method for the assay of the hypothetical non-androgenic substance. We decided to measure quantitatively the gonadotropin content of finely ground rat's pituitaries by the immature rat and mouse uterine weight response procedure in such animals.

Methods

Rats were used throughout as donors of pituitaries. Immature female rats and mice were used for assaying the gonadotropin content of the properly prepared pituitary suspensions.

After weighing each pituitary on a torsion balance immediately after removal, it was placed in an agate mortar with a small amount of solid carbon dioxide. When sufficient glands were collected in this way they were ground with the dry ice until a very finely divided and thoroughly mixed pulp was obtained. The pulp was next diluted with a definite volume of normal salt solution. Various dilutions were then made with normal salt solution and injected into 21 day old female rats or female mice a few days after weaning. In the same way fresh suspensions of pituitaries were made on two successive days and injected into the immature rats or mice. The rats were injected once per day, the mice twice per day for 3 days. Three and five or more different levels of pituitary suspensions were injected into 3 to 5 rats and mice respectively. The immature rats were sacrificed 24 hours after the last injection, the mice 48 hours after the last injection. The animals and uteri were weighed.

X-ray Sterilization of Male Rats

The animals were placed on a specially designed X-ray board equipped with leather straight jackets with adjustable metal cores which rendered the animals immobile. The board was then covered with a large lead plate with a single orifice which was adjusted to expose the testes only. Four rats were irradiated at one time.

A total of 36 male rats approximately 6 months of age were given 600 Roentgen units once a week for 4 weeks. After resting for 60 days for probable maximum development of hyperactivity of the hypophyses (10) the rats were sacrificed and the pituitaries assayed for gonadotropic activity by the method previously described.

Mature male rats of approximately the same age were castrated and allowed to rest for 60 days and then sacrificed for assay. Similarly the pituitaries of normal male rats were used

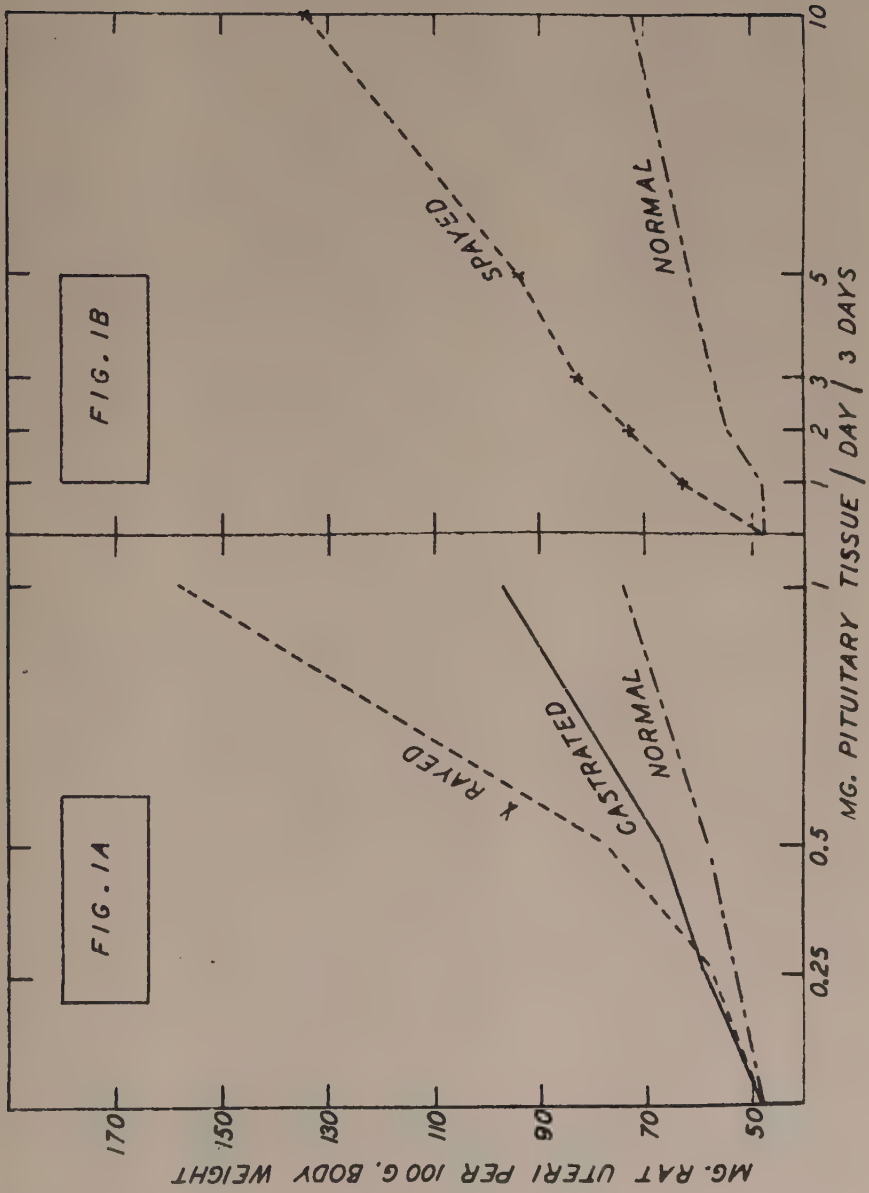
as controls. The results are shown in Table 1 and Figure I A. A total of 170 immature female rats were used in this series of experiments.

TABLE 1
STUDIES ON X-RAY STERILIZED RATS

Number of Rats	Treatment	Average Body Weight	Average Pituitary Weight	Average Testis Weight	Average Seminal Vesicle Weight	Average Prostate Weight
		grams	mg.	mg.	mg.	mg.
36	Testes X-rayed 2400 R. U.	286	8.5	867	935	300
15	Castrated	281	10.1		8	4
40	No treatment	292	8.0	2725	868	308

The most striking results of these studies are that the X-rayed sterilized rats' pituitaries developed a much higher gonadotropin content than those of the castrated litter mates and that there was no atrophy of the prostates or seminal vesicles in the X-ray treated animals. In fact, the average seminal vesicle weight in the X-ray treated donors was slightly higher than the average weight in the normal control animals. Histological examination of the testes revealed atrophy of the seminiferous elements while the interstitial cells were well preserved.

These results indicate that a hormone secreted by the germinal tissue of the normal testis has been lost and that this loss permits the pituitary to become richer in gonadotropin content and presumably also to secrete more actively. This hypothetical hormone is independent of the hormone or hormones secreted by the interstitial tissue and which maintain the normal prostate and seminal vesicle weights. This conclusion can be drawn since in the case of X-ray treated animals interstitial tissue remained intact and prostate and seminal vesicle weights were maintained. The slight increase in prostate and seminal vesicle weights over normal controls was probably due to the increase in gonadotropin secretion by the hypophysis. The fact that the X-ray sterilized animals yielded the most potent pituitaries suggests another possibility, namely, that the X-ray sterilization may have produced



a foreign toxic substance in the testes and thus produced the castration changes in the pituitary.

The Pituitaries of Spayed and Normal Adult Female Rats

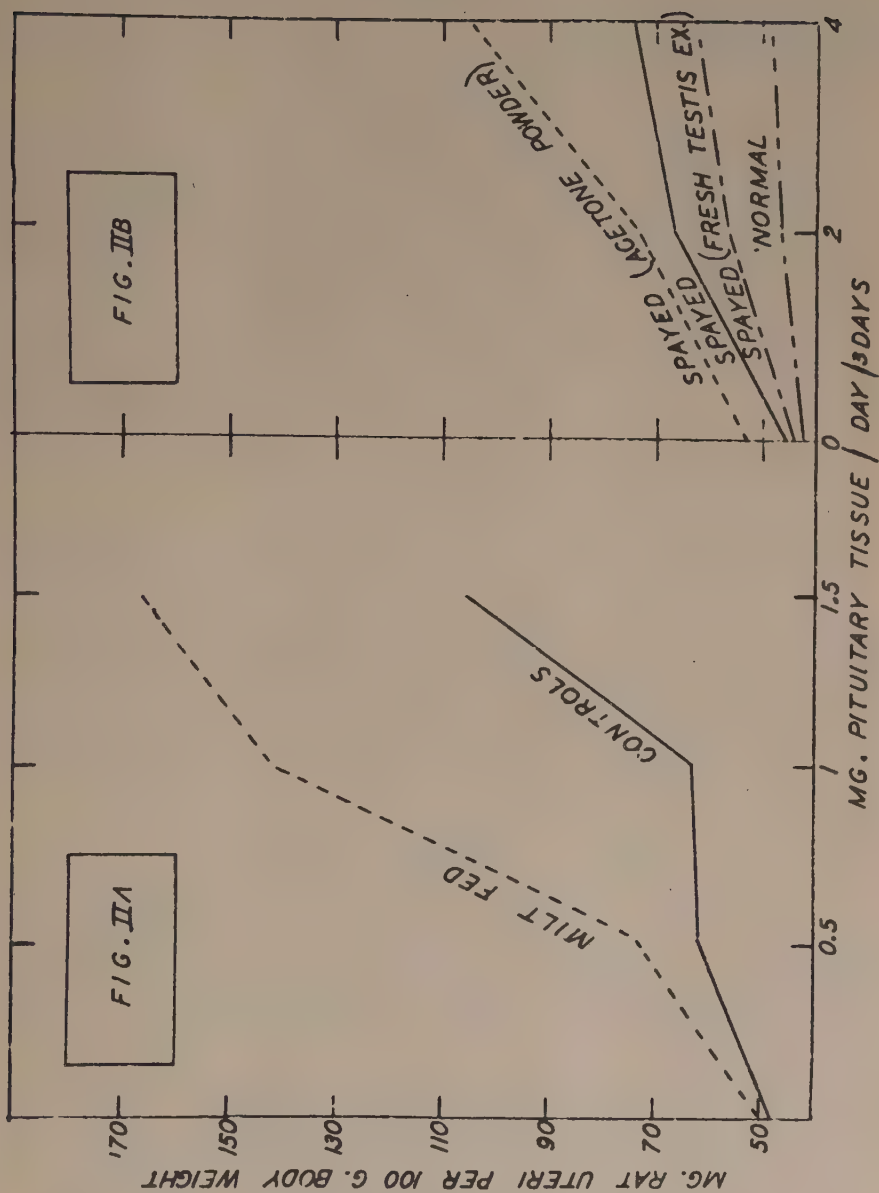
The graphs shown in Figure I B represent the average gonadotropin response in a series of experiments in which 62 spayed and 31 normal mature rats were used as donors.

Obviously removal of the ovary has qualitatively the same effect as castration or X-ray sterilization in the male. In the male castrate the pituitary is approximately twice as high in gonadotropin content as that of the normal male. In the female castrate the pituitary is approximately five times as active as in the normal female, but only approximately one-half as potent as in the normal male. In other words gonadectomy causes a greater per cent change in the gonadotropin content of the pituitary in the female than in the male, but in terms of absolute increases the castrated male's pituitary and especially that of the X-ray sterilized male surpasses those in the spayed female many fold.

The Effect of Feeding Salmon Milt to Castrated Rats

Male rats were castrated when three months old. Eleven were placed at once on a stock diet and eleven on a diet consisting of 50% stock diet and 50% canned salmon milt. After one month on these diets the pituitaries, adrenals, seminal vesicles and prostates were weighed and the pituitaries assayed as usual. The results on the assays are given in Figure II A.

The controls gave the usual values of castrated rats' pituitaries but milt feeding raised the gonadotropin content still more. In other words, milt feeding like X-ray sterilization or the injection of androgen free testis tissue is more effective in raising the gonadotropin content than simple castration. This may be one of the effects of a general toxic action. This is suggested by the loss in body weight. However, the absolute organ weights remained in the same ranges as in the control animals. If these organ weights are expressed as milligrams per 100 grams body weight then all gave higher values in the milt fed series, thus 90% in the adrenal, 53% in the seminal vesicle, 52% in the prostate and 35% in the pituitary. What the active component is



and what mechanism is involved remains to be determined. It should be added however that normal old male rats also lose some weight on the 50% salmon milt diet without seriously affecting the weights of the organs including the testes, suprarenals, seminal vesicle and prostate.

The Effect of Sulphanilamide in the Rat's Diet

Six young rats, 4 males and 2 females, were placed on a normal diet plus the addition of low doses of sulphanilamide. Six litter mates were kept on the normal diet. When complete sterility had developed in the animals receiving the sulphanilamide the pituitaries were assayed for gonadotropin activity. Immature mice were used for these assays. No significant differences in activities were observed. This study is incomplete because only a few animals were made available from another investigation in another laboratory. It should be repeated with the sexes segregated.

The Effect of a Vitamin E Deficient Diet

Ten mature female rats were kept on a vitamin E free diet for six months, after which time the animals' pituitaries were assayed as usual. An equal number of litter mates were tested in parallel. Complete sterility had developed in the animals kept on the vitamin E free diet.

No significant difference was found in the activity of the two sets of glands.

The Effect of Various Extracts on the Activity of the Rat's Pituitary

In this series spayed females were chosen as donors since they showed a greater per cent difference in pituitary hyperactivity from normal female controls than the normal and castrated males. Thirty-six mature female rats, approximately 6 months of age, were divided into four groups of nine each with appropriate distribution with reference to body weight and litter number. Group No. 1 was kept as normal controls. Group No. 2 was spayed, but received no other treatment. Group No. 3 was spayed and then injected subcutaneously twice daily for eight days immediately after the gonadectomy with testis tissue acetone powder. Group

No. 4 was spayed and injected subcutaneously twice daily for eight days with an aqueous suspension of fresh testis tissue.

The testes from 12 rats, 6 months old, were removed, weighed and placed in acetone after removing the tunica. After thorough mincing and extraction at room temperature with changes of acetone the tissue residue was next extracted at room temperature with benzene. The final tissue residue was allowed to dry at room temperature and then ground to a fine powder weighing 3.62 grams. This material was suspended in normal saline solution in amounts equivalent to 0.25 gram of the fresh testis tissue or 0.025 gram of the dry powder per ml. This was freshly prepared each day and 1 ml. injected subcutaneously twice daily.

The fresh testis tissue suspension for group No. 4 was prepared each day by suspending the very finely macerated testes from six months old rats in an equal weight of normal saline solution. The dose was 1 ml. per day.

For groups 2, 3 and 4 all operations and injections were staggered so that the injected donors and the spayed controls (group No. 1) were ready in groups on three successive days for the assays of the pituitaries. These assays also were staggered and carried out as usual.

The results are given in Figure II B.

On comparing Figures I B and II B it is seen that the pituitaries from the spayed untreated rats in II B are not as potent as in I B. This is no doubt due to the difference in the length of time elapsed since castration. It is however clear that injection of the suspension of fresh testis tissue caused an inhibition of the increase in potency of the pituitary tissue. This might be expected, but cannot be attributed to the androgen contained in the small amount of tissue and the short period of administration. The striking stimulation of gonadotropin potency by the acetone-benzene extracted tissue was just as much a surprise as the effect of X-ray sterilization in the male. This remains to be studied in detail before we can hope to arrive at an explanation.

This experiment was repeated with slight variations. Bull testis tissue acetone powder was used in place of the extracted rat testis. The bull testes were frozen with solid carbon dioxide at the abattoir immediately after removal. This material was ground thoroughly and transferred to a large volume of acetone.

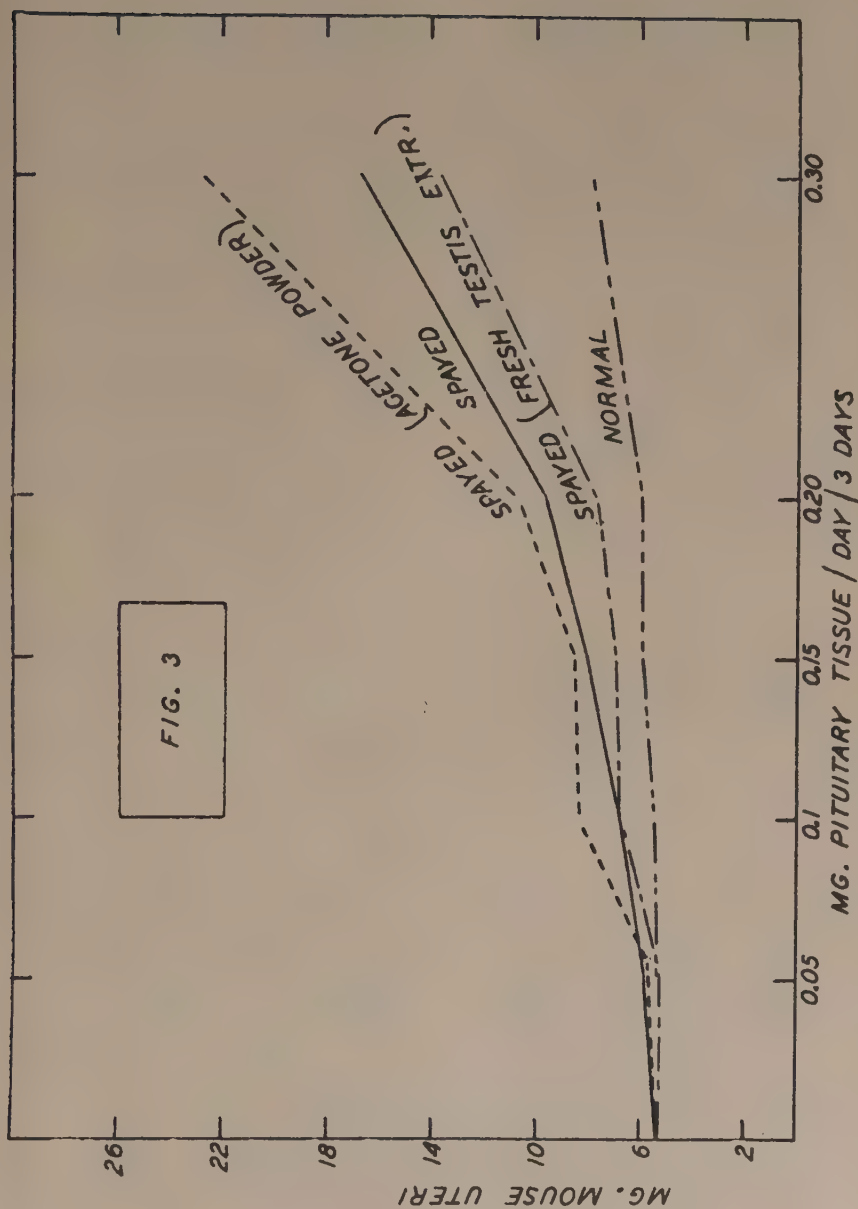
After thorough extraction with acetone at room temperature the material was dried at the same temperature and again ground in a mill. The benzene extraction was omitted.

Thirty-six mature female rats were divided into four groups of nine each from appropriately selected litter mates. Group No. 1 was kept as normal controls. Group No. 2 was spayed, but not treated otherwise. Group No. 3 was spayed and then injected subcutaneously twice daily for 8 days with 1 ml. of a saline suspension of the testis acetone powder containing the equivalent of 0.25 gram fresh testis per ml. Group No. 4 was spayed and injected twice daily with the freshly prepared suspension of fresh rat testis tissue. All operations and injections were staggered as in the previous experiment.

Mice were used for the gonadotropin assays. Since mice had been found to be much more sensitive than immature rats much lower doses of pituitary suspensions could be used in the final assays. Consequently the pituitaries were assayed at 0.05, 0.10, 0.15, 0.20 and 0.30 mg. per day for 3 days. Five mice were used for each level and ten were used as controls.

The results are given in Figure III. The preceding experiment is confirmed in every respect although bull testis tissue acetone powder was substituted for a corresponding rat testis preparation. We again conclude that the testis contains two non-androgenic substances which when injected into the spayed rat produce opposite effects on the gonadotropin content of the pituitary.

It is apparent that the inhibiting activity can be removed or destroyed by acetone extraction of the fresh testis tissue. From inspection of Figures II B and III it is obvious that if we are permitted to assume that acetone treatment has not produced an artefact, then the accelerating activity in the acetone powder is very potent and hence the inhibiting agent in the fresh tissue must also be very potent to overcome this accelerating action to the extent it has, namely to lower the gonadotropin content in the pituitaries even below those of the untreated spayed animals. It remains to be determined whether a synergistic phenomenon is involved in the action of the fresh tissue and whether the acetone soluble fraction retains the active inhibitor. It is very unlikely that the amount of androgenic material in the fresh testis emulsion is responsible for this inhibition. On the basis of androgen assays of extracts from rat testis tissue the amount of



androgen present cannot account for the inhibition. It is also very unlikely that it is due to the phospholipins in the aqueous acetone extract because the amount injected was very low.

A comparison of Figures I A, II B and III appears to justify the conclusion that X-ray sterilization results in the destruction of the tissue activity responsible for the secretion of the inhibiting agent, but not of those structures responsible for the production of androgens and the factors which stimulate the storage and/or overproduction of gonadotropins in the pituitary. The fact that injection of testis acetone powder and X-ray sterilization lead to the higher concentrations of gonadotropin in both cases suggests that the same mechanism may be involved. It will be very interesting to determine the action of the injection of suspension of fresh X-rayed rat testes and of acetone powders prepared therefrom. Many additional studies must be conducted on various fractions before we can say anything about the nature of the substances responsible for the acceleration and inhibition respectively. It is possible that both are widely distributed tissue constituents and that they may have nothing to do with the normal control of pituitary activity.

DISCUSSION

The results of these studies favor the dual or multiple endocrine activity hypothesis for the testis. Stimulation as well as inhibition of the gonadotropin content of the pituitary has been demonstrated. The effects of simple gonadectomy in either sex have been confirmed quantitatively. However, sterilization of the male by X-ray irradiation of the scrotum results in even more marked "castration changes" in the pituitary without interfering with the normal weights of the accessory sex organs. This suggests at least a dual mechanism in the control of the pituitary activity. Injection into spayed females of acetone powders prepared from rat or bull testicles or feeding salmon milts to castrated male rats also raises the gonadotropin of the pituitary. Whether this means increased storage and/or decreased secretion of gonadotropins remains to be determined.

The lowering of the gonadotropin content of spayed rats' pituitaries by treatment with saline suspensions of fresh rat testis tissue in small amounts cannot be explained at present. This

inhibition probably cannot be attributed to the androgens or estrogens found in these suspensions because the doses of pure androgens and estrogens required to produce a similar inhibition by other methods for its detection are 500 to 1000 fold those involved in the doses of fresh testis tissue used in this study. It is possible that a synergistic action or that some other forms of androgens or estrogens, such as various conjugated types or still others may be involved. There is no evidence for the presence of such hypothetical combinations in gonad tissue at present. Whether the lower gonadotropin content produced by fresh testis tissue suspensions is to be interpreted as a lowered production and/or a lowered storage in the pituitary remains to be determined.

The true interpretation and limitations of the assay method used in this study should be acknowledged. The method actually measures the secretion of estrogen in the immature rat or mouse as a result of the action of the gonadotropin complex. No claim is made that it measures only follicle stimulating activity. We are aware of the augmenting effects of the luteinizing factor in these preparations or of the luteinizing factor secreted by the normal immature rats or mice used in this method. At present we are limited to the purely comparative assays in normal animals by unfractionated pituitary tissue suspensions. It is to be hoped that more reliable and quantitative methods for the separation of these two gonadotropin factors and more sensitive and reliable assay methods for each factor may become available. If so, a better evaluation of our observations may be looked for.

SUMMARY

1. An accurate method for the estimation of the gonadotropin content of pituitary suspensions has been devised.
2. Normal and castrated male rats' pituitaries are more active in gonadotropin content than those from normal and spayed female rats respectively.
3. A salmon milt diet increased the gonadotropin content of the pituitaries of castrated male rats.
4. Vitamin E free diets or diets containing sulfanilamide had no significant effect in normal female rats.

5. The pituitaries from X-ray sterilized male rats are richer in gonadotropin content than those from castrated rats. In these X-ray treated rats the weights of the prostate and seminal vesicles did not fall but increased slightly.

6. Injection of saline suspensions of testis acetone powder into spayed rats causes an increased gonadotropin content in their pituitaries.

7. Injections of saline suspensions of fresh rat testis tissue into spayed rats lowered the gonadotropin content in their pituitaries but not to the normal level.

8. The nature of the substances involved in the stimulation and inhibition of the gonadotropin content of the pituitary and their distribution in other tissues as well as the physiological mechanisms involved in their actions remain to be determined.

9. The observations favor the dual or multiple function of the testis as an endocrine organ.

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